PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Marcin S. Filutowicz

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Examiner: Vanessa L. Ford

For: ANTI-MICROBIAL BIOTHERAPEUTIC AGENTS: ALTERNATIVES TO CONVENTIONAL PHARMACEUTICAL ANTIBIOTICS

File No.: 960296.00084

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner For Patents
Alexandria, VA 22313-1450

Dear Sir:

I, Marcin S. Filutowicz, on oath say and declare that:

1. I am the same Marcin S. Filutowicz who is the named inventor of the above-identified patent application. I am currently employed as a Professor of Microbiology at University of Wisconsin-Madison, a position that I have held since 1987. I obtained my Ph.D degree in Microbiology in 1979 from Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, and had my postdoctoral training with Dr. Donald Helinski, a prominent plasmid biologist at University of California-San Diego. I have worked as a research scientist specializing in the general area of microbiology for 28 years. For the last 22 years, my research has focused on plasmid biology (e.g., vegetative and conjugative DNA replication). I have published extensively in the area and have published many state-of-the-art review articles on this subject. Recently, I was invited to write a chapter on plasmid replication for a book titled "Plasmid Biology" to be published by the American Society of Microbiology. I was also invited to write a commentary on plasmid replication for Nature Structural Biology. A copy of my Curriculum Vitae is attached as Exhibit H.

2. I have reviewed the Office Action issued in this matter by the U.S. Patent and Trademark Office on July 29, 2003. I understand that Claims 1-12 and 16-27 are rejected for the alleged lack of enablement, in part because the Examiner believes that the specification does not provide enough evidence to demonstrate that the antibacterial agent is stable enough so that it can be successfully transferred from donor cells to recipient cells to kill the recipient cells. This Declaration is submitted to provide evidence that the antibacterial agents of the invention can successfully kill bacteria of the same species or of a different species as described in the specification.

3. At my direction, and under my supervision, members of my laboratory demonstrated in the manner described below that recombinant bacteria constructed according to the application successfully killed target bacteria of multiple species and strains, as was predicted in the patent application. The experiments describe below were based on the teaching of the application and involve only mature technology in the art of microbiology and molecular biology. Therefore, a skilled artisan can readily accomplish the experiments without undue experimentation.

4. In the first set of experiments, we constructed a plasmid called pir/pFL415 that contains γori as its origin of replication, oriT-RK2 as its origin of conjugative transfer, tetracycline and chloramphenicol resistant genes as its screenable markers, and a deletion of codons 106 and 107 of the pir mRNA as its run-away killing mechanism. A plasmid called pir/pFL129, that is the same as pir/pFL415 except that it does not contain the pir mRNA deletion, was used as a negative control. Another plasmid that is the same as pir/pFL129 except that its copy number is about 10 times higher was also used as a negative control (pir/pFL130). The above plasmids were each introduced into *E. coli* strain S17-1, which were the donor cells in which the replication of the plasmids were suppressed. Donor cells that contained pir/pFL129, pir/pFL130 and pir/pFL415 were designated as ECF0157, ECF0158 and ECF0715, respectively. These donor cells were then mixed and conjugated with recipient cells on a filter at either 30°C or 37°C. The killing of the recipient cells was then assayed by analyzing their growth on various antibiotic-containing media plate. As shown in Exhibits A, B and C, plasmid pir/pFL415 (provided in *E. coli* S17-1) was able to transfer and kill bacteria of the following species: *E. coli* K12-RL315 (Rif^r), *Serratia marcescens* (Rif^r) and *Salmonella enterica* (Kan^r).

5. In the second set of experiments, we constructed a self-transmissible plasmid that contains ori-RK2 as its origin of replication, oriT-RK2 as its origin of conjugative transfer, tetracycline resistant gene as its screenable marker, and a colicin gene, colE3, as its

mechanism for killing recipient cells. A plasmid that is the same as the above plasmid except that it is not self-transmissible was also constructed. A plasmid that is the same as the above self-transmissible plasmid except that it does not contain the colE3 gene was used as a negative control. The above plasmids were each introduced into *E. coli* strain S17-1, which were the donor cells in which the expression of the colE3 gene was suppressed. The donor cells were then mixed and conjugated with the recipient cells on a filter at 37°C for one to three hours. The killing of the recipient cells was then assayed by analyzing their growth on various antibiotic-containing media plate. As shown in Exhibits D, E, F and G, bacteria of the following species were successfully killed upon mixing with the donor cells: *E. coli* VH1000 (Rif^r), *E. coli* O157:H7 (Rif^r), *Salmonella enterica* serotype *typhimurium* (Rif^r), and *Pseudomonas aeruginosa* (S. West) (Rif^r).

6. In the experiments described above, mixing the donor calls and the recipient cells on a filter at either 30°C or 37°C led to the killing of the recipient cells. The same or similar conditions can be easily duplicated for animal feed. Thus, a skilled artisan can readily use the recombinant bacteria of the present invention to kill pathogenic bacteria in animal feed. No undue experimentation is required.

7. The art of microbiology has developed to a stage such that a skilled artisan can readily engineer a recombinant plasmid that can be transferred from a donor cell to and maintain stability in a recipient cell without undue experimentation. There may be rare examples in which this may be difficult to accomplish for various reasons. However, under most circumstances, this can be readily achieved by a skilled artisan. In fact, we and others have routinely created such plasmids and transferred them from donor to recipient cells.

8. At the very least, to practice the present invention for killing target bacteria, a skilled artisan can start with a plasmid that is natural to the target bacteria and genetically engineer the plasmid into a plasmid of the present invention. Further, a skilled artisan can use donor cells that are of the same species or strain as the target bacteria for hosting and transferring the recombinant plasmid. Thus, one only needs to transfer a recombinant plasmid derived from a natural plasmid of the target bacteria to the target bacteria from donor cells that are of the same species or strain of the target bacteria. This way, the stability issues that concerned the Examiner, i.e., stability in donor cells, stability during transfer, and stability in recipient cells (see Ambrozic et al., Klimke et al., Roberts et al, and Rahal et al.), should be minimized. The data provided in this Declaration confirms that the antibacterial agent of the present invention is stable enough to achieve the killing of target bacteria.

9. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated this 12 day of November.

Marcin S. Filutowicz

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